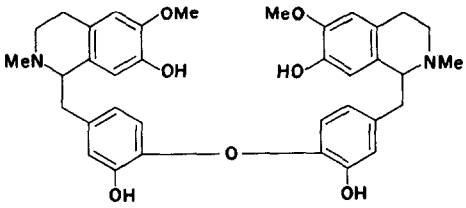

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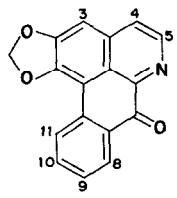

(2)

First, the crude alkaloid extract [4] from the plant was chromatographed on Si gel. The fraction eluted with C_6H_6–$CHCl_3$ (1:1) was recrystallized from MeOH to give liriodenine mp 275° (**2**) (CHN analysis, $[\alpha]_D$, IR, UV, MS, NMR). A small peak was observed at *m/e* 305 in the MS of crude liriodenine and the NMR showed an *O*-methyl group at 4·16 ppm as a tiny signal, suggesting the presence of a trace of a monomethoxy liriodenine, as an impurity [6]. The fraction eluted with $CHCl_3$ gave acetamide, mp 80–81° (IR, NMR). No other alkaloids were eluted from the column with $CHCl_3$–MeOH (99:1).

Secondly, the crude extract, according to Pallares and Garza's procedure [4]. The benzene-insoluble fraction, from which they isolated azequine, was extracted with dil. HCl and separation of phenolic and non-phenolic fractions was carried out in a usual manner. The non-phenolic fraction gave only liriodenine (**2**) in a small amount, but the phenolic fraction could not be purified because of its small quantity. When the whole fraction was methylated (CH_2N_2 in MeOH) there was no peak above *m/e* 400, in the MS suggesting that bisbenzylisoquinoline alkaloids were absent. The aqueous solution obtained during the extraction was treated with ammonium reineckate, but the precipitate of quaternary salts was not obtained in a sufficient amount. The benzene soluble fraction, after removal of acetamide by sublimation, was purified by chromatography on silica gel and again afforded only liriodenine (**2**).

Acknowledgement—We thank Dr. J. M. Muchowski, Syntex, Mexico, for his kind help for collection of natural product.

REFERENCES

1. Pallares, E. S. and Garza, E. M. (1948) *Arch. Biochemistry* **16**, 275.
2. Kametani, T., Iida, H., Shinbo, M. and Endo, T. (1968) *Chem. Pharm. Bull. (Japan)* **16**, 949.
3. Kametani, T., Shinbo, M., Fujikura, T., Kano, S. and Iida, H. (1967) *J. Pharm. Soc. Japan*, **87**, 753.
4. The crude extract obtained from the Yoloxochitl flower according to the Pallares and Garza's procedure [1] was donated by Dr. J. M. Muchowski whom we thank.
5. Buchanon, M. A. and Dickey, E. E. (1960) *J. Org. Chem.* **25**, 1389.
6. Taylor, W. I. (1961) *Tetrahedron* **14**, 42.
7. Warthen, D., Gooden, E. L. and Jacobson, M. (1969) *J. Pharm. Sci.* **58**, 637 (and refs. therein).

Phytochemistry, 1975, Vol. 14, pp. 1885–1888. Pergamon Press. Printed in England.

THE STRUCTURE OF ESCULENTIC ACID: A NEW TRITERPENE FROM *PHYTOLACCA ESCULENTA*

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Key Word Index—*Phytolacca esculenta*; Phytolaccaceae; new triterpene; esculentic acid.

Roots of *Phytolacca esculenta* van Houtte (Phytolaccaceae) have long been used as an indigenous medicine against edema and rheumatism. It was previously reported that sterols, sterol glucosides, acylated sterol glucosides, and terpenes, such as jaligonic acid and its methylester, phytolaccagenin were isolated from the roots [1, 2]. As mentioned in preliminary report [3], continuing study

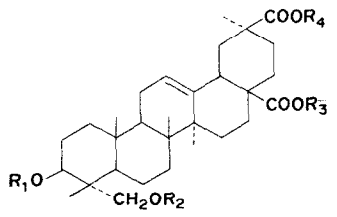
of the terpenoid constituents revealed an additional minor component, designated as esculentic acid. In the present paper, the complete structure and stereochemistry of esculentic acid is described.

Esculentic acid (**1**), $C_{30}H_{46}O_6$, mp > 360°, $[\alpha]_D^{25}$ +85·6° (*c* = 0·23 in EtOH), λ_{max}^{EtOH} 204 nm (log ε, 3·7), forms a dimethyl ester (**2**), mp 151–153°, a diacetate (**3**), mp 103–106°, and a dimethyl diacetate (**4**), mp 100–103°. The acid (**1**) gives a pink coloration in the Liebermann–Burchard test. The presence of one double bond in **1** is shown by positive tetranitromethane color test and by the consumption of nearly one mole of perbenzoic acid by **4**. Oxidation of **4** with CrO_3–HOAc furnishes an α,β-unsaturated ketone (**7**), mp 197–199°, λ_{max}^{EtOH} 249 nm (log ε, 3·73), and SeO_2 oxidation in HOAc leads to heteroannular diene (**8**), mp 77–79°, λ_{max}^{EtOH} 243, 251, and 260 nm (log ε, 4·02, 4·09 and 3·88, respectively). On the basis of the above experiments, **1** appears to be a dihydroxy-olean-12-ene dioic acid.

The ester **2** does not react with HIO_4, but on treatment with acetone in the presence of an acidic catalyst it affords the corresponding acetonide (**5**), mp 265–266°. These results indicate that **1** has no vicinal glycol group. On pyrolysis with Cu catalyst at 290° **1** gives HCHO, a typical reaction of the 3:23-diol system in the triterpene series [4–6], identified as its dimedone derivative, mp 187–189°.

The NMR spectrum (60 MHz in $CDCl_3$) of **4** shows 5 tertiary methyl signals at δ = 0·72 (3H), 0·83 (3H), 0·98 (3H), 1·12 (3H), and 1·14 (3H); 2 acetyl signals at 2·02 (3H) and 2·06 (3H); two methyl ester signals at 3·57 (3H) and 3·69 (3H); a multiplet centered at 5·37 (1H) due to an olefinic proton; a broad signal centered at 4·8 (1H) due to a proton on a carbon atom bearing a secondary acetoxyl group; and an AB quartet at 3·72 and 3·88 (1H each, *J* 12 Hz) due to 2 methylene protons on a carbon atom bearing a primary acetoxyl group, attached to an asymmetric center, C-4. The chemical shift attributed to the methylene protons is within the range for an equatorial CH_2OAc [7], and the broad signal attributed to the proton on C-3 is in rather higher field [7–9]. This evidence support the orientation of the two hydroxyl groups at C-3 β and C-23.

The highest C-methyl signal (δ = 0·72) appears upfield from 0·775 and one (δ = 3·57) of 2 methyl ester signals is upfield from 3·595, thus suggesting



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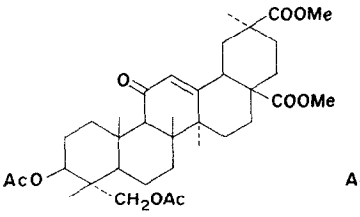

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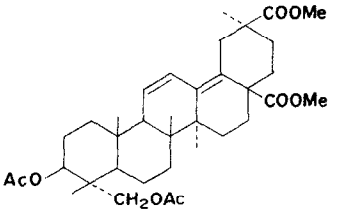
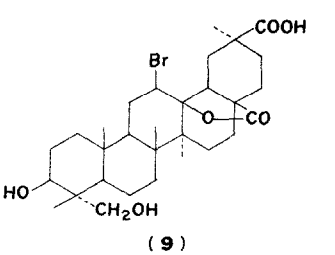


(8)

	R_1	R_2	R_3	R_4
(1)	H	H	H	H
(2)	H	H	Me	Me
(3)	Ac	Ac	H	H
(4)	Ac	Ac	Me	Me
(5)	—C(Me)$_2$—		Me	Me
(6)	H	H	Me	H



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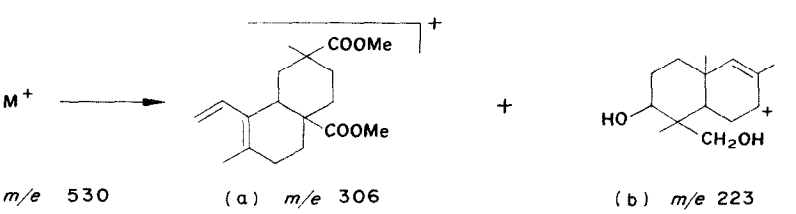

m/e 530 (a) *m/e* 306 (b) *m/e* 223

Table 1. NMR C–Me frequencies of esculentic acid derivatives

Structure	Observed and calculated values (Hz) Me-24	Me-25	Me-26	Me-27	Me-29	Me-30
For dimethylesculentate diacetate (**4**)						
Calcd.						
30-COOMe compd.	51·5	58·5	43	67·5	68	
29-COOMe compd.	51·5	59·5	43·5	67·5		75·5
Found	49·5	58·5	43	67	68	
For dimethylesculentate acetonide (**5**)						
Calcd						
30-COOMe compd.	62·5	60·5	42	69·5	68·5	
29-COOMe compd.	62·5	61·5	42·5	69·5		76
Found	62	57	42	68	68	

Frequencies relative to TMS measured in $CDCl_3$ at 60 mHz.

the presence of a C-28 carboxyl group [8]. This was confirmed by the formation of a bromolactone (**9**), mp 231–233°, 1767 cm^{-1} (γ-lactone). On saponification with 10% MeOH–KOH for 8 hr **2** furnishes a monomethyl ester (**6**) mp 306–307°, in 83% yield. On further saponification with KOH in ethylene glycol **6** gives **1**. This differential behaviour of the two carbomethoxyl groups towards saponification would suggest the presence of the less hindered ester group. The molecular ion M^+ 530 (1·6%) is in agreement with the molecular formula $C_{32}H_{50}O_6$. The two peaks at *m/e* 306 (**a**) (49%) and 223 (**b**) (9%) represent the retro-Diels–Alder fragmentation at 12:13 double bond. Fragment **b** is not too abundant as expected [10] and ion **a** accommodates two COOMe. Loss of elements of two COOMe and two H from ion **a** gives rise to peaks at *m/e* 247 (50%), 246 (66%), 187 (100%), and 186 (64%). This fragmentation pattern is identical to those of methyl esters of jaligonic and spergulagenic acid, which contain both 28- and 30-carboxylic acid [1, 11].

The recent data [12–14] on the influence of substitution on the methyl frequencies in the NMR spectrum of 12-oleanene derivatives were applied in order to elaborate the orientation of the carboxyl group at C-20 in **1**. It was found that the observed values in Hz of the chemical shifts for C-methyl groups of **4** and **5** are in better agreement with the values calculated for 30-carbomethoxy structure than with the values for the corresponding 29-carbomethoxyl structure Table 1). These data together with the facts that saponification rate of **2** is similar to those of dimethylesters of jaligonic and spergulagenic acid [1, 15], and that treatment of **1** with Ac_2O and $HClO_4$ does not give an anomalous acetate [16–18] but a normal diacetate [1, 11], support the β (axial, i.e. C-30) rather than the α (equatorial, i.e. C-29) orientation of the carboxyl group at C-20. Consequently, the structure and stereochemistry of esculentic acid is established as 3β, 23-dihydroxy-olean-12-ene-28, 30-dioic acid (**1**).

EXPERIMENTAL

Plant. Raised from seeds collected in the wild in the vicinity of Seoul, and grown in experimental garden. Voucher specimen is deposited in the Herbarium of this Institute.

Extraction. Homogenized fresh roots were extracted with Et_2O. The Et_2O layer was partitioned with saturated $NaHCO_3$. Ppts formed by adding *d*-HCl were chromatographed over SiO_2. Elution with $CHCl_3$–MeOH (30:1) gave **1** (Found: C, 71·82; H, 9·25. $C_{30}H_{46}O_6$ requires: C, 71·68; H, 9·22%).

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REFERENCES

1. Woo, W. S. (1971) *J. Pharm. Soc. Korea* **15**, 99; (1973) *Lloydia* **36**, 326.
2. Woo, W. S. and Kang, S. S. (1973) *J. Pharm. Soc. Korea* **17**, 152, 161; (1974) **18**, 203.
3. Woo, W. S. (1974) *J. Pharm. Soc. Korea* **18**, 229.
4. King, F. E., King, T. J. and Ross, J. M. (1954) *J. Chem. Soc.* 3995.
5. Anantaraman, R. and Pillai, K. S. M. (1956) *J. Chem. Soc.* 4369.
6. Ayengar, K. N. N. and Rangaswami, S. (1966) *Tetrahedron Letters* 1947.
7. Kubota, T., Tonami, F. and Hinoh, H. (1967) *Tetrahedron* **23**, 3333.
8. Shamma, M., Glick, R. E. and Mumma, R. O. (1962) *J. Org. Chem.* **27**, 4512.
9. Shibata, S., Kitagawa, I. and Fujimoto, H. (1965) *Tetrahedron Letters* 3783.
10. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
11. Chakrabarti, P., Mukherjee, D. K., Barua, A. K. and Das, B. C. (1968) *Tetrahedron* **24**, 1107.

12. Tursch, B., Savoir, R., Ottinger, R. and Chiurdoglu, G. (1967) *Tetrahedron Letters* 539.
13. Cheung, H. T. and Williamson, D. G. (1969) *Tetrahedron* **25,** 119.
14. Woo, W. S. and Kang, S. S. (1974) *Korean J. Pharmacog.* **5,** 69.
15. Chakrabarti, P., Mukherjee, D. K. and Barua, A. K. (1966) *Tetrahedron* **22,** 1431.
16. King, F. E. and Morgan, J. W. W. (1960) *J. Chem. Soc.* 4738.
17. Tursch, B., Leclercq, J. and Chiurdoglu, G. (1965) *Tetrahedron Letters* 4161.
18. Thiessen, W. E., Levy, H. A., Dauben, W. G., Beasley, G. H. and Cox, D. A. (1971) *J. Am. Chem. Soc.* **93,** 4312.

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*N-ISO*BUTYLOCTADECA-*TRANS*-2-*TRANS*-4-DIENAMIDE: A NEW CONSTITUENT OF *PIPER GUINEENSE**

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Key Word Index—*Piper guineense*; Piperaceae; dienamide; *N-iso*butyloctadec-*trans*-2-*trans*-4-dienamide.

Piper guineense, West African Black Pepper or Ashanti Pepper, is a woody climber widely distributed throughout West Africa [1]. The fruits have been used as a flavorant while preparations of leaves, roots and seeds have been used internally as medicinal agents for the treatment of bronchitis, gastrointestinal disease and upset, venereal disease, and rheumatism [1]. Externally, preparations of the seeds have been used for their counterirritant and insecticidal properties [1].

P. guineense is reported to contain the lignans (+)-sesamin and (+)-ashantin [2, 3] but no other compounds have been isolated. *Iso*butylamides have to date been reported mainly in the Compositae [4] and Rutaceae [5]. However, *N-iso*butyldeca-*trans*-2-*trans*-4-dienamide has recently been isolated from the seeds of *Piper sylvaticum* Roxb. [6, 7]. This paper reports the isolation and identification of *N-iso*butyloctadeca-*trans*-2-*trans*-4-dienamide, a new naturally occurring *iso*butylamide from *P. guineense*.

Extraction of the seeds with acetone afforded an oily extract which was partitioned between Et_2O and HCl (1%). The Et_2O layer was shaken with $NaHCO_3$ (2%) and subsequently chromatographed over silicic acid. Elution with petrol–$CHCl_3$ (1:1) afforded *N-iso*butyloctadeca-*trans*-2-*trans*-4-dienamide as needles, m.p. 78–80°. The UV spectrum showed λ_{max}^{MeOH} 260 nm ($\log \epsilon$ 4·47) and was indicative of a sorbic (2,4-hexadienoic acid) chromophore [8]. The IR spectrum: (**1**) (M − Me_2CH, 8%); (**2**) (M − $NHCH_2CHMe_2$, 66%); (**3**) (M − $C_{12}H_{25}$, 22%); and (**4**) (M − $C_{13}H_{27}$, 30%) indicated the presence of a N–H group (3295 cm^{-1}), an $\alpha,\beta,\gamma,\delta$-conjugated carbonyl (1622 cm^{-1}) and a *trans–trans* conjugated diene system (1652 and 995 cm^{-1}) and was therefore suggestive of an $\alpha,\beta,\gamma,\delta$-unsaturated secondary amide (–C=C–C=CO–NH–). The MS showed M^+ at *m/e* 335 (100%) for $C_{22}H_{41}NO$ and other important fragments at 292 (M–Me_2CH, 8%) (**1**), 263 (**2**), 180 (**3**), and 166 (**4**). NMR: δ 0·93 (9H, m), CH_3; 1·30 (22H, br, *s*) CH_2; 1·65–2·20 (3H, m) CHÆ and CH_2 next to conj. double bond; 3·25 (2H, *t*, J=6 Hz) CH_2–N: 5·20–6·10 (4H, m) NH and CH=; 6·90–7·40 (1H, m) CH in centre of conj. system. The

* Part 6 in the series 'Constituents of West African Medicinal Plants'. For Part 5, see Tackie, A. N., Dwuma-Badu, D., Dabra, T. T., Knapp, J. E., Slatkin, D. J. and Schiff, P. L., Jr. (1974). Accepted for publication *Experientia*.